ARTICLE ADDENDUM

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Evolutionary routes from a prebiotic ANA-world

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Recent experimental support has been generated for a model of prebiotic development that postulates a role for Amyloid-Nucleic Acid (ANA)-fibers as the earliest replicating entities capable of undergoing Darwinian evolution. Here, this new model is compared with existing RNA-world models with a particular focus on trajectories that lead to evolutionary-beneficial interactions between nucleic acid, protein and lipid components. This analysis suggests a number of new areas for fruitful experimental studies.

The ability of RNA to both store information and carry out enzymatic reactions links genotype to phenotype within a single molecule and has led to the idea that life initiated as an 'RNA-world.'[1] Implicit in this model is the idea that RNA molecules later evolved, through selection, the ability to recruit biomolecules including protein, lipids and DNA.[2] In our recent paper, we presented data supporting an alternative model for prebiotic development in which the earliest replicating entities were amyloid-nucleic acid (ANA)-fibers.[3]

Here probable evolutionary trajectories from an ANA-world will be compared with RNA-world alternatives with a focus on how growth, replication and division are achieved.[4] Alternative 'metabolism-first' models will not be discussed as they do not, as yet, include clear mechanisms that allow Darwinian inheritance.[5,6] The comparisons will highlight key mechanistic differences and should help focus future efforts to reconstruct 'living' systems from prebiotic components.

Growth

In an early ANA-world, sequence-independent electrostatic interactions between prebiotically-generated nucleic acids and peptides generate ANA-fibers (**Fig. 1A**). Growth is driven by the recruitment of basic amyloidogenic peptides through interactions with uncompensated nucleic acid negative charges at fiber ends. By contrast, growth in an RNA-world centers on the increase in length of RNA molecules driven by RNAzymes with sequence-specific ligase or polymerase activities.[4,7] A compelling trajectory from the RNA-world to a cellular world involves the encapsulation of RNA molecules in liposome vesicles. In these 'protocells' RNAzymes replicate nucleic acids with which they share a compartment, while nucleic acid polymerisation could also drive the growth of the vesicle by increasing its osmotic pressure.[4,8,9] In summary, growth in an RNA-world would be driven by nucleic acid polymerisation, while growth in an ANA-world would be driven by protein fiber elongation.

Selection for growth in an ANA-world would involve the selective elongation of ANA-fibers whose associated nucleic acids were better able to incorporate amyloidogenic peptides (elongase activity). One hypothetical scheme is illustrated in **Figure 1B** that suggests how this selection could culminate in the formation of the ribosome.[2] A key early step would be the emergence of fiber-associated nucleic acids that preferentially recognized amyloidogenic peptides (e.g., those with alternating hydrophobic/ hydrophilic residues). If the supply of prebiotically-generated peptides were limiting, competition would lead to the selection of RNAzymes that ligate or extend shorter peptides. Experimental studies have identified RNAzymes that aminoacylate ribonucleic acids and synthesize short peptides.[10-12] However, less work has been done on RNAzymes that bind peptides. It is interesting to speculate that the strong hydrophobic/

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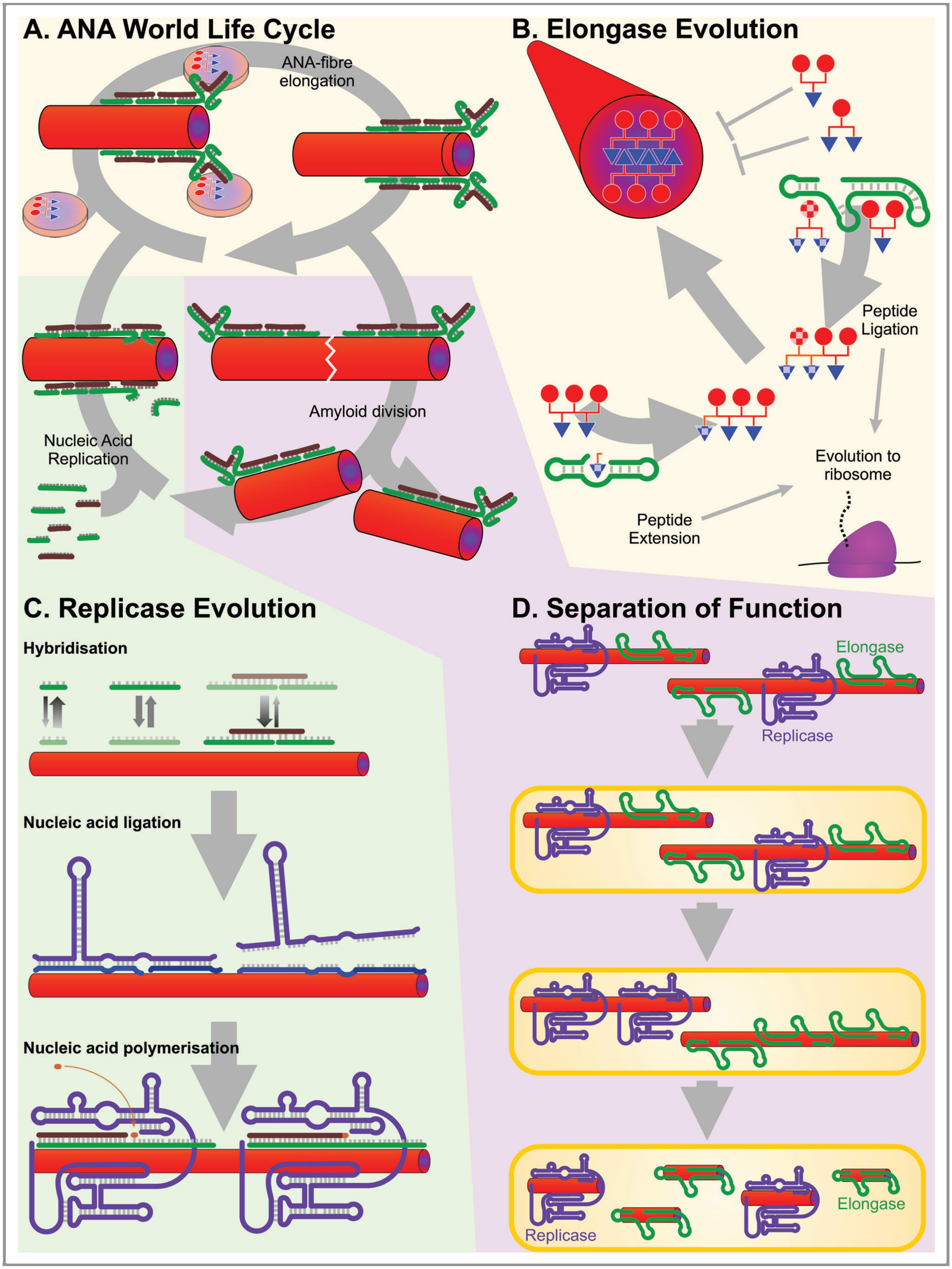
A. ANA World Life Cycle
ANA-fibre elongation
Amyloid division
Nucleic Acid Replication
B. Elongase Evolution
Peptide Ligation
Evolution to ribosome
Peptide Extension
C. Replicase Evolution
Hybridisation
Nucleic acid ligation
Nucleic acid polymerisation
D. Separation of Function
Elongase
Replicase
Replicase
Elongase

Figure 1. (See previous page). Evolutionary routes from a prebiotic ANA-world. (A) ANA world life cycle. Nucleic acids recruit short basic amyloidogenic peptides to the growing end of the amyloid fibril primarily through charge interaction with the phosphate backbone. With increasing length, the amyloid fibrils become vulnerable to hydrostatic forces, eventually causing them to shear. This generates daughter fibrils that inherit associated and therefore related nucleic acids. The amyloid fibril promotes nucleic acid replication by enhancing hybridization. Inter- and intra-molecular hybridization allows nucleic acids to adopt secondary structures, some of which may enhance the incorporation of amyloidogenic peptides. Further selection for nucleic acids that promote amyloid elongation or replication could evolve ribosome-like or polymerase/ligase-like activities respectively. (B) Elongase Evolution. Only peptides of the sufficient length with an alternating pattern of hydrophobic (triangle) and hydrophilic (circles) residues are incorporated into the amyloid fiber (red). Under conditions where peptides of sufficient length (e.g., 6 residues) are unavailable, RNAzymes associated with the fibril generate suitable peptides by ligating or extending shorter ones. RNA-specific recognition of suitable amino acid sequences evolves into the ribosome (purple). (C) Replicase Evolution. Initially, the amyloid promotes hybridization and double-strand formation from short overlapping oligonucleotides. The resulting longer strands provide a higher charge density and therefore associate more strongly with the fiber to enhance its elongation. Evolution of nucleic acid ligase or polymerase activities enhances the quantity and length of associated nucleic acids under conditions of limiting polynucleotides. This process advances through selection to generate protein fiber-associated nucleic acid polymerases. (D) Separation of Function. Initially, both elongase and replicase RNAzymes are required to be on a single fiber. With the incorporation of ANA fibers within membrane vesicles, the elongase and replicase functions could evolve on different fibers since the importance of the fiber for compartment formation would be removed.

hydrophilic conservation in triplet codon specificity may be inherited from an ancient elongase activity that functioned to generate amyloid fibers. By contrast, in the RNA-world or in an RNA-containing protocell, it is unclear what selective advantage would accrue from the evolution of RNAzymes with amino-acid conjugation or peptide polymerisation capabilities.

Replication

The key feature of replication in the earliest ANA-world is base pairing of short prebiotically-generated oligonucleotides (**Fig. 1A**). As shown in our recent study, amyloid fibers would aid replication by concentrating nucleic acids and by enhancing their hybridization.[3] By contrast, replication in the RNA-world requires an RNAzyme replicase with a specific 3-dimensional structure that can synthesize copies of itsself. Thus in the RNA-world, one type of molecule serves two functions (growth and replication), while in the ANA-world, two molecules come together (protein and nucleic acid) to provide the same functions.

In the early RNA-world, selective advantage would accrue to molecules that have an enhanced ability to copy themselves. Within liposome 'protocells' RNAzyme replicases would copy neighboring (related) templates using RNA ligase and/ or polymerase activities. Recent experimental studies have shown that RNAzyme ligases can drive an RNA-only chain reaction.[13] Equivalent polymerase RNAzyme chain reactions have not yet been demonstrated nor have chain reactions been demonstrated inside liposome 'protocells' where RNAzyme substrates might be limiting.

In an ANA-world, greater efficiency of the interlocking ANA-fiber cycles shown in **Figure 1A** would result from the evolution of RNAzyme replicases that can copy both themselves and elongase molecules (**Fig. 1C**). A key experimental test for this trajectory would be to demonstrate that RNAzymes (e.g., nucleic acid/ protein ligases and polymerases) remain functional when associated with ANA-fibers.[7,14,15]

Division

Division in an ANA-world involves the shearing of long ANA-fibers into daughter fibers that inherit related nucleic acids (**Fig. 1A**). By contrast, division in the RNA-world involves the separation (by denaturation) of replicase RNAzymes from newly generated copies. Division of RNAzyme-containing protocells would, like ANA-fibers, be driven by hydrostatic shear producing daughter protocells that inherit related nucleic acids.[16] A potential problem for ANA-fibers comes from the observation that amyloid fibers tend to aggregate. This clumping would tend to reduce the genetic uniqueness of individual fibers. However, aggregation may be reduced if ANA-fibers interact with fatty acids (**Fig. 1D**).

The incorporation of ANA-fibers into a protocells could reduce clumping and may ultimately allow the separation of replicase and elongase RNAzyme activities as shown in **Figure 1D**. Growth of liposome-encapsulated ANA-fibers would occur both through increased osmotic pressure and through fiber-associated forces at the growing fiber tips. It is interesting to note that protein fibers play an on-going role in the growth and division of archaea and bacteria,[17,18] although modern fibers involve protein-protein interactions that can be better regulated than the amyloid cross-β motif.

Preliminary data from our group suggests that fatty acids, like nucleic acids, promote the formation of amyloid fibers and aggregates from short peptides. We suggest that a prebiotic 'triple' mix of lipids, short peptides and nucleic acids would have generated a range of charge-based aggregates including liposome-encapsulated ANA-fibers, RNAzyme-containing 'protocells' and fatty acid-peptide aggregates that could act as a feed for the growth of ANA-fibers. The further study of inter-compartmental interactions should be a fruitful area to test predictions from these distinct models.

Conclusion

A feature of the ANA- and RNA-world evolutionary trajectories is the importance of interactions between nucleic acids (NA), protein (P) and lipid (L) components in the prebiotic environment. In the ANA-world, we suggest that the NA-P interaction generated the first replicating entities capable of NA-based Darwinian evolution. In the RNA-world, NA replication alone is proposed to precede others, while the 'protocell' model expands this to a world based on NA-L interactions. The transition from an NA, NA-L (protocell) or NA-P (ANA-world) to the 'modern' NA-L-P world may have been a relatively late development that followed the establishment of Darwinian selection, but may alternatively have been necessary for its earliest implementation.

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